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SERUM ANTIBODIES TO HUMAN ENTERIC CORONAVIRUS-LIKE PARTICLES IN AUSTRALIA, SOUTH AFRICA, INDONESIA, NIUE, AND PAPUA NEW GUINEA

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Summary. — Relatively high levels of antibody to human enteric coronavirus-like particles were detected in the sera from rural Aborigines in Australia. Levels were generally much lower in the sera from urban Aborigines, and extremely low to not detectable in the sera from Europeans. Antibody to coronavirus-like particles was also detected in the sera from rural blacks from South Africa, in the sera from Indonesia and Niue, and also possibly in the sera from rural villagers from Papua New Guinea, but in the latter case at only very low level.

Key words: *human enteric coronavirus-like particles*; *serum antibody*

Introduction

The work of Gerna *et al.* (1985) and Resta *et al.* (1985) has confirmed the initial finding of Caul *et al.* (1975) and established the existence of human enteric coronaviruses resembling the classical coronaviruses. However, there have been numerous other reports, prior to and since these, of the frequent observation in stools of coronavirus-like particles (CVLPs) apparently morphologically dissimilar to the classical coronaviruses (MacNaughton and Davies, 1981; Schnagl *et al.*, 1987), and to the toroviruses (Schnagl *et al.*, 1987). The causal association of such particles with human disease has yet to be established. Particles of this type have often been observed to be excreted as frequently or even more frequently by individuals without diarrhoea when compared to those with it (Schnagl *et al.*, 1979; McNaughton and Davies, 1981; Sitbon, 1985).

Characterization of these particles has proved difficult mainly due to their inability to be grown in culture, except in perhaps one or two cases (Caul and Egglestone, 1977; MacNaughton and Davies, 1981). Recently it was shown that CVLPs of this type in Australia were not coronaviruses or duodenal brush border vesicles (Schnagl *et al.*, 1987), but what type of infectious agent they might be, or whether they or other such particles are in fact infectious agents has also still to be determined. However, some support for the con-

sideration of such particles as infectious agents has been provided by Schnag *et al.* (1986), with their finding of an excellent correlation between the ex cretion rates of CVLPs and the serum antibody levels to CVLPs in the po pulations they studied in Australia.

In this report we extend the preliminary observations of Schnagl *et al* (1986) on the prevalence of antibody to CVLPs in Australia. As well w report for the first time the finding of serum antibody to CVLPs in Soutl Africa, Indonesia, and Niue, and also possibly in Papua New Guinea, althoug only at very low level in this case.

Materials and Methods

Sera. Sera were obtained in 1986 from male and female Aborigines, living in essentially rura areas throughout the state of Western Australia, who had been referred to the State Healt Service for a variety of reasons. The individuals ranged in age from 3 months to 76 years. A well sera were obtained from Europeans from towns throughout Western Australia who ha also been referred to the State Health Service in 1986. Generally randomly collected sera wer also obtained from male and female Aborigines and Europeans from urban areas of several sta capital cities of Australia. These were collected between 1983 and 1988 and the ages of the indi viduals ranged from 2 months to 73 years, with the Aborigines and Europeans being age- an sex-matched.

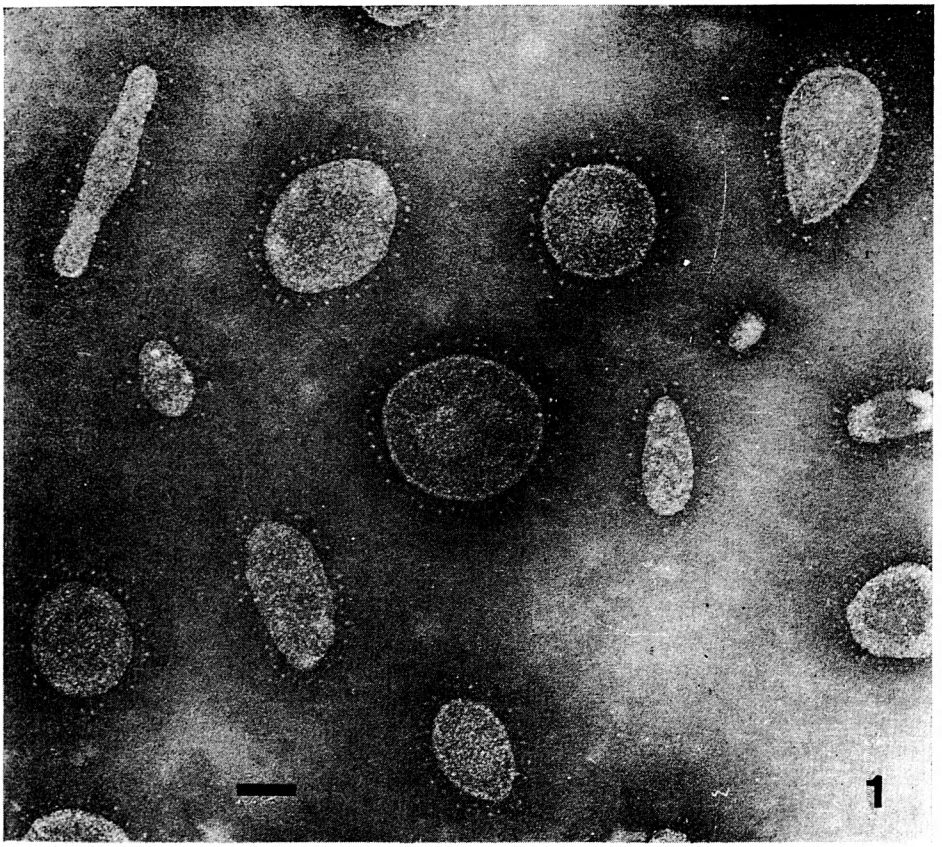
The South African sera tested were kindly provided by Professor B. D. Schoub, Institute fo Virology, University of the Witwatersrand, Johannesburg. They were collected in 1985 fro black individuals ranging in age from 8 months to 76 years, living in rural areas ranging fro Namibia to northern and eastern Transvaal. The Indonesian sera were obtained through th courtesy of Dr R. F. Bishop, Department of Gastroenterology, Royal Children's Hospita Melbourne, Australia and were collected in 1978–1979 from individuals from varying socio economic backgrounds from the Jogjakarta area. The individuals ranged in age from 2 month to 83 years and a number of them had been admitted to hospital in Jogjakarta for a variety o reasons. M. Dometrakakis, Virology Laboratory, Fairfield Hospital, Melbourne, Australia, kindl supplied the Niue sera, which came from individuals ranging in age from 9 months to 17 year and had been collected in 1982. The Papua New Guinea sera were kindly provided by Dr. R. San ders, Papua New Guinea Institute of Medical Research, Goroka. They had been collected in 198 and 1982 from rural villagers ranging in age from 4 to 75 years from highland and lowland region of the Eastern Highlands Province. In all of the above cases approximately equal numbers o sera were obtained from males and females.

Methods. For use in the determination of decoration antibody titres CVLPs were purifie as outlined previously (Schnagl *et al.*, 1987). Briefly, particles were first clarified by centrifugatio at 1,300 × g for 20 min, and after centrifugation of the supernatants at 100,000 × g for 1 h the resultant pellets were layered onto a 10–50% (v/v) angiografin (Schering AG, Berlin) gradien in phosphate buffered saline. The gradients were centrifuged at 75,000 × g for 6 hr and CVL containing fractions further purified by centrifugation in a 30% (v/v) glycerol/50% (w/w) potas sium tartrate continuous gradient at 150,000 × g for 17 hr. Such purified particles are shown i Fig. 1.

For the determination of serum antibody titres to CVLPs the immune electron microscopi decoration method outlined previously (Schnagl *et al.*, 1986) was used. Briefly, a loopful o

Fig. 1. Highly purified preparation of CVLPs as used for immune electron microscopy fro a glycerol/potassium tartrate gradient. Note the pleomorphic particles surrounded by cha acteristically shaped surface projections or spikes. The thin stalks of the surface projection are not readily visible on a number of the particles.

Fig. 2. Antibody (decoration) from a serum sample covering the surface projections (spikes) o CVLPs. The bars represent 100 nm.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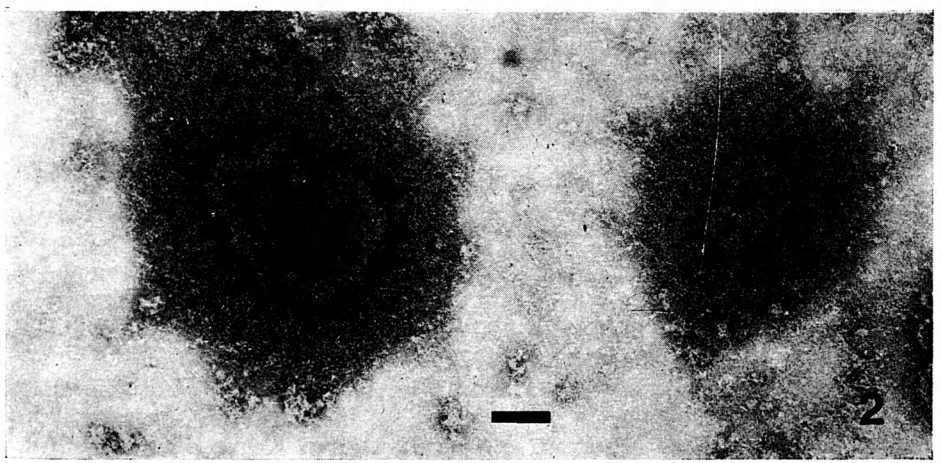
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Table 1. Ranges of antibody titres to CVLPs in sera from Australia, South Africa, Indonesia, Niue and Papua New Guinea

Origin of sera (Number tested)	Range of decoration antibody titres to Central Australian CVLPs
Australia	
Rural Aborigines from throughout Western Australia (25)	50—1000
Europeans from towns throughout Western Australia (15)	<2—2
Urban Aborigines from state capital cities (25)	<2—100
Urban Europeans from state capital cities (31)	<2—5
South Africa	
Rural blacks from Namibia to Transvaal (20)	5—200 (10—500)*
Indonesia	
From the Jogjakarta area (41)	2—200
Niue (Pacific Ocean) (20)	10—100
Papua New Guinea	
Rural indigenous villagers from highlands and lowlands (50)	<2—10

* Range of titres obtained using South African CVLPs for the half of the sera able to be tested with these.

purified CVLPs was applied to a formvar and carbon coated electron microscope grid and the excess removed after one minute. Before the grid had time to dry it was floated on 10 µl of appropriately diluted serum and maintained in a humidifier at room temperature for 30 min. Excess fluid was then removed and the grid stained for electron microscopy with one tenth saturated ammonium molybdate. The decoration titre was taken as the reciprocal of the highest dilution of serum at which antibody (decoration) was found to be attached to at least one of 50 or 100 observed particles (Fig. 2).

Results and Discussion

The ranges of decoration antibody titres to CVLPs obtained with the various sera tested are shown in Table 1. All of the titres were obtained by testing against CVLPs from Central Australia, except in the case of the South African sera where South African CVLPs were also used.

Titres obtained with the sera from rural Australian Aborigines from throughout Western Australia were similar to those obtained previously and since from Aborigines from Central Australia, generally well over 1,000 km

to the east (Schnagl *et al.*, 1986; Schnagl and Foti, 1988, unpublished data). Similar titres were obtained whether purified Central Australian or Western Australian CVLPs, which proved to be antigenically identical, were used. There was no difference between males and females in the prevalence of high and low titres, as had been found previously in Central Australia (Schnagl *et al.*, 1986). It was found that antibody titres to CVLPs in sera from urban Aborigines, from the several Australian state capital cities, were generally very much lower than those from the rural Aborigines (Table 1).

CVLP antibody titres in European sera were either extremely low or there was no measurable titre (Table 1). In fact there was only one serum from amongst the 46 such sera tested where a titre of above 2 was recorded, and this was a titre of 5. Further it is felt that considering the concentration of serum involved that a titre of 2 is not necessarily one that could be considered with confidence as being positive for CVLPs (Schnagl *et al.*, 1986).

It has been known for some time that CVLPs were excreted by a substantial percentage of individuals in the rural black population of at least some areas of South Africa including the Transvaal (Schoub, 1981). It was therefore of interest to test sera from rural South African blacks for the presence of antibody to such particles in the light of the results obtained with Australian Aborigines. Using Central Australian CVLPs titres ranging from 5—200 were obtained (Table 1), but enough South African CVLPs (from Lesotho) were available to enable half of the South African sera to be tested with them. The titres thus obtained were generally approximately twice as high as those obtained with Central Australian CVLPs, giving a range in titres of 10—500. This finding of higher titres using the South African CVLPs is not surprising as these CVLPs had previously been shown to differ antigenically from the Central Australian ones (Schnagl *et al.*, 1987).

Decoration antibody titres to CVLPs in sera from individuals from the Jogjakarta area of Indonesia ranged from 2—200 (Table 1). It is unfortunate that Indonesian CVLPs were not available to more completely test these sera as the titres obtained were in approximately the same range as those obtained with the South African sera and Central Australian CVLPs. Higher, more indicative titres may have resulted from the use of Indonesian CVLPs. It is interesting to note with the Indonesian sera that the majority giving higher titres (of 50—200) were from females, 11 of 20, compared to 1 of 21 for males.

It had previously been found that up to 66% of individuals on the Pacific island of Kiribati were excreting CVLPs (R. D. Schnagl, unpublished data), and as sera from Kiribati were not available it was decided to test sera from the Pacific island of Niue. Living conditions on the two islands are very similar. Using Central Australian CVLPs a range of titres of 10—100 was obtained with these sera (Table 1), indicating at least a moderate level of antibody to CVLPs, especially when compared to the results obtained with sera from Australian Europeans.

CVLPs had previously been found to be excreted by individuals from the same province, although it is not known if some of these were from the same

villages, from which the Papua New Guinea sera were obtained (R. F. Bishop, personal communication). In the light of the results obtained with the Aboriginal sera across Australia, and the South African sera, it is surprising that antibody titres to CVLPs in the Papua New Guinea sera were so low (Table 1). The majority were <2 or 2, with only a few being higher, up to 10. Unfortunately Papua New Guinea CVLPs were not available for testing as it would have been of interest to determine if there was a substantial antigenic difference between the Papua New Guinea CVLPs from the areas under study and the Central Australian particles.

With regard to the antibody titres to CVLPs in Australian sera it is again clear that in general by far the highest titres were evident in the population with the highest excretion rates for the particles. The excretion rates for CVLPs in rural Australian Aborigines have ranged up to 85% (Schnagl *et al.*, 1978; Schnagl *et al.*, 1979), whereas excretion rates for such particles in Central Australian Europeans have ranged up to 18% (Schnagl *et al.*, 1979), and in urban Europeans were found to be less than 1% (Schnagl *et al.*, 1986). Further, antibodies to CVLPs have now been detected in Aborigines across Australia, not just in one specific area. It may be that the lower CVLP antibody levels found in urban Aborigines very possibly reflect their generally better living conditions.

Evidence of antibodies to CVLPs has now also been found in sera from several other countries. However, it is clear that caution may be required in interpreting the levels determined if only a single source of CVLPs is used in testing sera from different countries. Lower titres could be obtained as a result of antigenic differences between CVLPs from different areas. Over all the Australian and South African results do provide further support for the consideration of the CVLPs dissimilar to the classical coronaviruses as infectious agents or parts of infectious agents, but more definitive proof is still required.

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References

Caul, E. O., and Egglestone, S. I. (1977): Further studies on human enteric coronaviruses. *Arch. Virol.* **54** 107–117.

Caul, E. O., Paver, W. K., and Clarke S. K. R. (1975): Coronavirus particles in faeces in patients with gastroenteritis. *Lancet* **i**, 1192.

Gerna, G., Passarani, N., Battaglia, M., and Rondanelli, E. G. (1985): Human enteric coronaviruses: antigenic relatedness to human coronavirus OC43 and possible etiologic role in viral gastroenteritis. *J. infect. Dis.* **151**, 796–803.

MacNaughton, M. R., and Davies, H. A. (1981): Human enteric coronaviruses. Brief review. *Arch. Virol.* **70**, 301–313.

Resta, S., Luby, J. P., Rosenfeld, C. R., and Siegel, J. D. (1985): Isolation and propagation of a human enteric coronavirus. *Science* **229**, 978–981.

Schnagl, R. D., Brookes, S., Medvedec, S., and Morey, F. (1987): Characteristics of Australian

human enteric coronavirus-like particles: a comparison with human respiratory coronavirus 229E and duodenal brush border vesicles. *Arch. Virol.* **97**, 309–323.

Schnagl, R. D., Greco, T., and Morey, F. (1986): Antibody prevalence to human enteric coronavirus-like particles and indications of antigenic differences between particles from different areas. *Arch. Virol.* **87**, 331–337.

Schnagl, R. D., Holmes, I. H., and MacKay-Scollay, E. M. (1978): Coronavirus-like particles in Aboriginals and non-Aboriginals in Western Australia. *Med. J. Aust.* **1**, 307–309.

Schnagl, R. D., Morey, F., and Holmes, I. H. (1979): Rotavirus, coronavirus-like particles, bacteria and parasites in Central Australia. *Med. J. Aust.* **2**, 115–118.

Schoub, B. D. (1981): Enteric adenoviruses and rotaviruses in infantile gastroenteritis in developing countries. *Lancet* **ii**, 925.

Sitbon, M. (1985): Human-enteric-coronavirus-like – particles (CVLP) with different epidemiological characteristics. *J. med. Virol.* **16**, 67–76.